Resistance to bendiocarb and malathion, and the Gly12Ser mutation in the *ace1* gene of *Aedes aegypti* from Tapachula

Ramsés Alejandro Cuautle-Hernández[1], R. Patricia Penilla-Navarro[1], Karla Saavedra-Rodríguez[2], Francisco Solis-Santoyo[1] and Américo D Rodríguez[1]

1. Instituto Nacional de Salud Pública, Centro Regional de Investigación en Salud Publica, Tapachula, Chiapas, México
2. Departamento de Microbiología, Universidad Estatal de Colorado. Fort Collins, Colorado, USA.

Summary

The use of organophosphates and carbamates has increased in Mexico due to resistance to pyrethroids in Aedes aegypti, so it is necessary to monitor the emergence of resistance mechanisms to these insecticides. The susceptibility to bendiocarb and malathion (CDC) was determined in mosquitoes from four neighborhoods in Tapachula, Chiapas. Resistance ratio (RR) was calculated by comparing the LC50 of wild mosquitoes with those of a susceptible strain. Overexpression of enzymes that could metabolize these insecticides, as well as the detection of insecticide insensitive acetylcholinesterase (iAChE) were assessed by WHO biochemical tests; while the presence of the Gly12Ser mutation in the ace-1 gene encoding acetylcholinesterase was determined by rtPCR. Three colonies of Ae. aegypti (Bonanza, Paraíso and Benito Juárez) showed a RR greater than 10 for bendiocarb, while in those from Palmeiras was 2.91. However, the Palmeiras's mosquitoes scored the highest RR (3.68) and the highest resistance for malathion. Esterases, monooxygenases, and glutathione S-transferases enzymes were overexpressed in mosquitoes from the four neighborhoods, while iAChE presented heterogeneous levels. The Gly12Ser mutation was found in all mosquito homogenates that had or did not have propoxur-insensitive AChE, as well as in dead and alive mosquitoes exposed to bendiocarb. Therefore, the presence of the Gly12Ser mutation in ace-1 of Ae. aegypti, could not be considered as a mechanism of resistance to carbamates and organophosphates in the collected mosquitoes from Tapachula. However, insensitivity of AChE to propoxur was detected by biochemical assays, suggesting that a mutation in the ace1, not identified yet, is present. Therefore, even though low levels of resistance to bendiocarb or malathion have been recorded in these colonies of mosquitoes, the monitoring of iAChE in those mosquito populations is mandatory.

Objetives

Objetive 1. Estimate the resistance to bendiocarb and malathion in *Ae. Aegypti* from four neighborhoods in Tapachula.

Objetive 2. Quantify the levels of enzymes that metabolize the insecticides bendiocarb and malathion.
Objetive 3. Analyze the frequency of the Gly12Ser mutation in live mosquitoes exposed to bendiocarb and malathion.
Objetive 4. Determine the association of enzymatic activity and the Gly12Ser mutation in mosquitoes resistant to bendiocarb and malathion.

Methodology

1. Biological assay

We worked with mosquito eggs from four neighborhoods in Tapachula whose previous results showed high resistance parameters for both bendiocarb and malathion.

The selected sites were the Paraíso, Palmeiras, Bonanza and Benito Juárez neighborhoods. These sites present a suburban habitat with breeding sites on insecticide-treated surfaces.

As a reference strain during the development of the bioassays and biochemistry, Ae. aegyti from the susceptible strain New Orleans.

The diagnostic doses defined by CDC for bendiocarb (12.5 µg / mL) and malathion (50 µg / mL) were used.

Five 250 mL Wheaton bottles (with screw cap) were used per bioassay: four bottles impregnated with insecticide and one impregnated only with the solvent used as control.
Three repetitions were carried out for each insecticide with its diagnostic dose, thus placing 15 mosquitoes per bottle, 75 were exposed in each bioassay, resulting in 225 for each insecticide from each mosquito neighborhood. 1800 mosquitoes from the four field mosquito neighborhoods and 1800 mosquitoes from the laboratory susceptible colony were exposed to the two insecticides.

Resistance ratio (RR) was calculated by comparing the LC50 of wild mosquitoes with those of a susceptible strain.

Knockdown/dead, and alive mosquitoes were recorded and then frozen for enzyme assays and rtpcr.

2. Enzyme quantitative assays

Biochemical assays were undertaken to evaluate the enzyme activity in the field mosquitoes compared against that of the susceptible New Orleans colony according to Penilla et al, 1998

1. Acetylcholinesterase activity inhibited by insecticides
2. Esterase concentrations
3. Gluthation S-transferase activity
4. Cytochromes P450 content
5. Protein content

3. Molecular assays

DNA was extracted from each mosquito following the salt extraction technique (Bender et al., 1983).

Amplification for the ace-1 fragments was carried out in a BIORAD® real-time thermal cycler, using the following primers designed by Karla Saavedra-Rodríguez, from Colorado State University.

We used the melting curve designed by P.h. Karla Savedra which helped us identify the presence of the mutation Gly12Ser in the ace1 gene of Ae. Aegypti which helped us to identify susceptible homozygous, mutant heterocytogous and susceptible homozygous

Results

To evaluate resistance to malathion and bendiocarb, four of the 26 neighborhoods of mosquitoes with higher resistance ratios were used according to the results obtained by the work team of the research project "Insecticide resistance management to preserve pyrethroid susceptibilitiy in *Aedes aegypti*" during 2018. The selected mosquito neighborhoods were Bonanza and Paraíso with resistance to bendiocarb, Palmeiras with resistance to malathion and Benito Juárez with resistance to both insecticides.

Three colonies of Ae. aegypti (Bonanza, Paraíso and Benito Juárez) showed a RR greater than 10 for bendiocarb, while in those from Palmeiras was 2.91. However, the Palmeiras's mosquitoes scored the highest RR (3.68) and the highest resistance for malathion.

Neighborhood	RR Bendiocarb	RR Malathion
Benito Juárez	10.30	3.90
Bonanza	9.98	1.46
Paraíso	11.15	1.31
Palmeiras	2.91	3.68

Figure 1. Resistance factors (RR) to bendiocarb and malathion in mosquitoes collected in four neighborhoods of Tapachula (results obtained by the work team of the research project "Insecticide resistance management to preserve pyrethroid susceptibility in Aedes aegypti" during 2018

In the results of the bioassays, significant levels of resistance to bendiocarb were detected. In the bioassays of the mosquitoes exposed to malathion, no survivors were presented because they did not recover from the phosphorylation of the insecticide, so these results showed that there is a susceptibility to the organophosphate malathion, however, the ezymes show of the onset of resistance based on insecticide metabolism.

Individuals insensitive to AChE were observed associated with resistance to organophosphates and carbamates in the neighborhoods Benito Juárez tuvo un 51%, Bonanza 29%, Palmeiras 71% y Paraíso 12% indicating the presence of a resistance mechanism in AChE, as has been found in other mosquito species such as Culex and Anopheles.

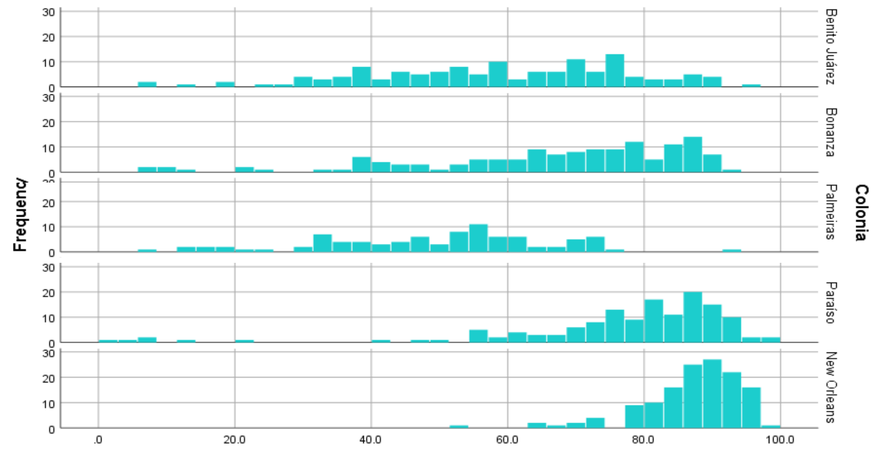

Figure 2. Percentage inhibition of AChE by Propoxur

p<0.05

Enzyme	% de inhibition of AChE		α esterase		β esterase		ρNPA		GST		P450		RR benidocarb	RR malathion
Neighborhood	N	X̄	N	X̄	N	X̄	N	X̄	N	X̄	N	X̄		
Benito Juárez	134	58.8 ***	140	0.00073	139	0.00046	140	0.1173 ***	139	0.682 ***	91	0.0013 ***	10.3	3.9
Bonanza	137	63.3 ***	140	0.00092 ***	137	0.00053	140	0.1410 ***	133	0.8587 ***	139	0.0022 ***	9.98	1.46
Palmeiras	90	49.5 ***	141	0.00073	140	0.0004 *	141	0.1164 ***	127	0.6542 ***	132	0.0013 ***	2.91	3.68
Paraíso	139	76.6 ***	141	0.00093 ***	141	0.00056 *	141	0.1277 ***	141	0.7642 ***	63	0.0009 ***	11.15	1.31
New Orleans	136	87.1	140	0.00071	139	0.00051	140	0.0671	137	0.1773	73	0.0005		

*** P<0.001, **P<0.01, *P<0.01

Figure 3. Results of biochemical tests where propoxur-insensitive AChE and enzymes were significantly elevated in the four wild Tapachula colonies compared to the New Orleans reference strain and its resistance factors to bendiocarb and malathion

On the other hand, resistance to organophosphates is also caused by increased esterase synthesis, which affects the effectiveness of the insecticide, thereby reducing the number of insecticide molecules that manage to reach the target site. It was observed that the mosquitoes from the Bonanza and Paraíso neighborhoods showed an increase in the production of α esterase and in the case of β esterases only Paraíso presented a higher quantity. Regarding the ρNPA esterases, a significant overexpression was observed in the mosquitoes of the four colonies.

In this study, the Gly12Ser mutation was detected in both resistant and susceptible mosquitoes. However, it was not possible to obtain an association between the presence of the Gly12Ser mutation and individuals resistant to bendiocarb in mosquitoes from the Paraíso neighborhood when compared with the susceptible mosquitoes from the Palmeiras neighborhood.

When we tried to associate the levels of AChE inhibition and the Gly12Ser mutation, mosquitoes with the mutant genotype were obtained both in individuals with a low inhibition percentage and in those with a high inhibition percentage. Therefore, there is no association between the insensitivity of AChE and the presence of the Gly12Ser mutation in these four mosquito populations collected in Tapachula.

Porcentaje de inhibición de AChE	Colonia	GLY GG	SER/GLY AG	SER AA	
>60%	Palmeiras	0	6	17	$P = 0.10$
<60%	Palmeiras	0	13	14	
>60%	Paraíso	0	16	16	$P = 1$
<60%	Paraíso	0	9	9	

Figure 4. Association between percentage of AChE inhibition and genotype obtained from the Gly12Ser mutation of 100 mosquitoes from the Palmeiras and Paraíso neighborhood.

Conclusion

Mosquitoes from Benito Juárez, Bonanza and Paraíso neighborhoods presented RR> 9 indicating moderate levels of resistance to bendiocarb, while the mosquitoes from the Benito Juárez and Palmeiras neighborhoods presented a RR <4 to malathion, indicating that they are still susceptible to malathion. according to WHO parameters.

The biochemical assays demonstrated that wild mosquito populations have developed resistance mechanisms based on the metabolism of the main families of insecticides.

The mosquitoes of the Paraíso neighborhood had all the enzymes analyzed overexpressed, indicating their role in the detoxification of bendiocarb.

No association was identified between bendiocarb and malathion resistant individuals and propoxur insensitivity of AChE.

The frequencies of the Gly12Ser mutation were similar between the mosquitoes of the Paraíso and Palmeiras colonies.

The Gly12Ser mutation in the ace-1 gene of Ae. aegypti from Tapachula was not associated with individuals resistant to bendiocarb and malathion, as well as with mosquitoes with an insensitivity of AChE to propoxur.

Alout, H., Berthomieu, A., Hadjivassilis, A., & Weill, M. (2007). A new amino-acid substitution in acetylcholinesterase 1 confers insecticide resistance to Culex pipiens mosquitoes from Cyprus. Insect Biochem Mol Biol, 37(1), 41-47. doi:https://doi.org/10.1016/j.ibmb.2006.10.001

Fournier D. (2005). Mutations of acetylcholinesterase which confer insecticide resistance in insect populations. Chemico-biological interactions, 157-158, 257–261. https://doi.org/10.1016/j.cbi.2005.10.040

Penilla R. P., Rodríguez A. D., Hemingway J., Torres J. L.., Arredondo-Jimenez J. I. and Rodriguez M. H. 1998. Resistance management strategies in malaria vector mosquito control. Baseline data for a large-scale field trial against Anopheles albimanus in Mexico. Med Vet Entomol. 12:217-233.